Beyond the Black Queen Hypothesis.

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Running title : *The Black Queen Hypothesis and beyond*

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Abstract| The **Black Queen Hypothesis** (**BQH**), recently proposed to explain an evolution of dependency based on gene loss, is gaining ground. This paper focuses on how the evolution of dependency transforms interactions and the community. Using Agent Based Modeling we suggest that species specializing in the consumption of a common good escape competition and therefore favor co-existence. This evolutionary trajectory could open the way for novel long-lasting interactions and a need to revisit the classically-accepted assembly rules. Such evolutionary events also reshape the structure and dynamics of communities, depending on the spatial heterogeneity of the common good production. Let Black be the new black!

Popular among theories of ecology and evolution, the Red Queen Hypothesis (Van Valen, 1973) has recently been echoed by a new hypothesis: the Black Queen Hypothesis (BQH, Morris *et al*., 2012) (Figure 1) which concerns the evolution of dependency between organisms.

While the Red Queen Hypothesis sets the basis for (mostly antagonistic) co-evolution, the BQH renews and puts into perspective current understanding of the evolution of interactions between free-living organisms within microbial communities. This novel reductive theory describes evolutionary mechanisms potentially leading to the connectedness between organisms in a community. More precisely, it provides theoretical interpretations on the evolution of dependencies through adaptive gene loss in free-living organisms.

In the BQH context, some free-living organisms called beneficiaries "avoid" having a function in order to optimize their adaptation to the environment. This loss of function is made possible because other organisms in their close environment (helpers) publicly and continuously provide for the function, offering a (partially) stable environment. The mechanism underlying this particular loss of function is genome reduction through gene loss. This type of evolutionary process is of major significance in the context of long-lasting interactions, as beneficiary species develop strong dependency on the function provided by helper species. The fact that most bacterial species cannot be grown in monocultures, referred to as the "*uncultured microbial majority*" (Giovannoni *et al*., 2014), could result from such dependency-based genes loss, as the species are unable to grow when extracted from their community.

After its introduction, the Black Queen Hypothesis was echoed in papers describing the evolution of organisms through adaptive functions and genes loss (Ellers *et al.,* 2012; Giovannoni 2012; D'souza *et al.,* 2014; Giovannoni *et al.,* 2014; Luo *et al*., 2014), the evolution of interactions (Estrela *et al*., 2012; Hussa *et al*., 2013), notably cooperation (Sachs et

Hollowell, 2012) and evolution of the community (e.g., Sachs et Hollowell 2012; Mitri *et al*., 2013; Hanson *et al*., 2014). It also triggered one dedicated evolutionary experiment (Morris *et al*., 2014) and uncovered new possibilities regarding the outcomes of other evolutionary experiments (D'souza *et al*., 2014, Hosoda *et al*, 2014).

One notable point of the BQH is the association of adaptive genome reduction with free-living organisms (i.e. organisms living independently of any host), a phenomenon which had not been apparent before. This evolutionary event of adaptive genome reduction stems from a particular type of interaction: the use of a common good (i.e. a freely available element present in the environment) (Figure 1).

Being a recent hypothesis, the BQH has not been thoroughly tested. Nevertheless we focused on the development of new ideas related to this hypothesis. We detail herein the possible mechanisms driving the observed loss of genes, and then use a modeling approach to fathom the consequences of this adaptive genes loss on population dynamics, at the community scale and in different ecological contexts. Thus our aim was to explore the BQH beyond its original definition. A corollary of the BQH is also introduced.

Size matters in the Black Queen Hypothesis.

A key element in this hypothesis is the loss of genes, which expresses a selected genome reduction. While evolution is usually associated with genome complexification (Wolf and Koonin, 2013), in the BQH the source of evolutionary opportunities is simplification through genes loss. Typically, gene loss results from two different forces: genetic drift and positive selection.

Genetic drift or positive selection for gene loss in the BQH?

Genetic drift refers to changes in allele frequencies of a population due to random sampling. The ability of drift to influence allele frequencies is inversely proportional to population size.

By definition, natural selection increases fitness whereas genetic drift operates at random, and only occasionally confers patent fitness benefits.

If a function becomes useless, the selection pressures on genes involved in that function are lifted. In the absence of purifying selection acting on these genes, circum-neutral mutations can accumulate (McCutcheon and Moran, 2011) and be randomly retained by genetic drift in a small population, leading to the decay and eventual loss of these genes. Given the large size of the populations encompassed by the BQH, genetic drift is excluded as the main driver of evolution (Morris *et al*., 2012). One example of the BQH concerns the most abundant photosynthetic organisms on Earth: *Prochlorococcus* (Partensky *et al*., 1999).

General genome reduction & removal of specific gene

Gene loss is known to be selected via two distinct operating modes: it can be favored by (i)general genome reduction, and (ii)the removal of specific targeted gene(s). The two systems most likely act together.

Positive selection for genome reduction refers to genome streamlining *sensu stricto* (Ochman & Moran, 2001; Giovannoni *et al*., 2005) , which is defined as the selection process that […] *acts to reduce genome size because of the metabolic burden of replicating DNA with no adaptive value* [...](Giovannoni *et al*., 2005). Indeed, every function has a constitutive cost, considering both genomic content and metabolism (Giovannoni *et al*., 2005, Lahti *et al*., 2009; Kreft & Bonhoeffer, 2005; Driscoll *et al*., 2011) but this cost is usually offset by the fitness benefits the function provides(Giovannoni *et al*., 2014). If a function loses its beneficial effects it will eventually be purged to reduce energy costs. Because retaining a bigger genome is costly (maintenance, replication, regulation), positive selection for genome reduction is assumed to be the main driver of genome reduction, at least in oligotrophic environments(Giovannoni *et al*., 2014; Dufresne *et al*., 2005; Hottes *et al*., 2013). A reduction or elimination of redundant

and useless genetic material will occur from one generation to the next. Consistently, free-living microorganisms are reported to often experience genome reduction via a loss of paralogues for multi-copy genes (Porter & Crandall, 2003).

Thus in the genome streamlining theory (Ochman & Moran, 2001; Giovannoni *et al*., 2005), selection favors gene loss, as smaller genomes provide more adaptive advantages than bigger ones.

Recent experimental tests of evolutionary dynamics have shown that it may not be genome reduction itself, but particular and individual gene loss that confers the greatest advantages (D'souza *et al*., 2014, Cooper *et al*., 2001; Lee & Marx 2012, Pande *et al*., 2013), especially if the cell's lack of metabolite production is compensated by the habitat (D'souza *et al*., 2014). The fitness gained from a given gene deletion is dependent on its metabolic function (e.g., which metabolite it codes for) and on the position of the deletion in the metabolic pathway. For example, the deletion of genes at the end of a bio-synthetic pathway could be more advantageous than the deletion of anterior genes (D'souza *et al*., 2014). Thus the energy costs linked with anabolism could be efficiently reduced by the removal of specific targeted genes.

Specialization towards common goods consumption

A special interaction: the circulation of a common good

Every species in a community is linked to one or several other species, thereby forming an intricate web of direct and indirect interactions, metaphorically described as a tapestry in which the weaving (*i.e.,* interactions between species) is as important as the species themselves (Estes *et al*., 2013).

In the BQH (Morris *et al*., 2012) species evolutionary dynamics are based on indirect interactions through *common goods* utilization. Common goods are freely available elements

present in the environment for both the producing species and other species around. The producers of common goods can still have preferential access to these goods (Estrela *et al.,* 2015), thereby avoiding the emergence of a "tragedy of the commons" situation (Hardin, 1968). Thus, the interaction between "helper" and "beneficiary" species is a case of indirect symbiosis existing through the flux of a common good, and the evolution of interactions between the two species can be considered as a side-effect of common good consumption.
Within the community, helper individuals transform their microbial vicinity into a stable and homogeneous place (by continuous production of a common good) allowing beneficiary mutant organisms to follow this adaptive path of specialization.

The rise of the mutant

To further understand the Black Queen's dynamics we used an agent-based model (ABM, supplementary information). We based our simulations on one or two species, then we introduced a mutant with higher fitness than its ancestor in order to observe (i) the temporal and spatial patterns of its invasion in the community (ii) the dynamics of the simulated organisms.

These simulations showed that when a fitter mutant unable to synthesize the common good emerges in a population (i.e. a loss-of-function mutant) this new strain will supplant its ancestors. But if the ancestors are the only helpers around, the beneficiary mutant population never excludes its original population due to its vital dependence on the production of the common good (Figure 2a). Thus the BQH also fits for a single species model. Nevertheless, if another species in the community produces enough of the common good, the mutants (because they are fitter) will replace the ancestors(Figure 2b).

In our simulations, the helpers' population is always sustained and their density (Supplementary Information) reaches a steady state (Figure 3a). The dependence of the

beneficiaries on the helpers forces both populations to be in equilibrium. This confirms the BQH prediction (Morris *et al*., 2012) whereby a loss-of-function mutant will be able to expand within its ancestral population if the function loss gives the mutant a growth advantage over its ancestors but the mutant retains a need for the common good.

Helper or Beneficiary?

What, in a community, determines which species will evolve into a beneficiary or into a helper? The current proposition by Morris *et al*. (2012) is that beneficiaries are species that evolve the most rapidly. We propose a new idea.

Our simulation reveals that when the minimum value of a common good needed for the mutants to multiply increases, it lowers the density of the mutants at steady state, while it increases the density of the helper species(Figure 3b). This is tied to the fact that increasing the need for a common good increases the dependency of the mutants on helper species producing this common good. As dependency is constraining, we suggest that the fittest of the loss-of-function mutants will be the one on which a rise in dependency will have the least effect, i.e. for which the loss of function is less enslaving. Nevertheless, if the function is accessory, it could be entirely lost by the community, and the hypotheses would no longer stand, suggesting that the BQH might only be valid for functions vital to the organisms. We suggest that a potential loss-of-function mutant may be a species with a 'silently advantageous' trait, which will only fully express its benefits after a concomitant mutation. Such a trait could be a minor need for the common good or a faster consumption rate for example.

Thus, in species adopting a beneficiary trajectory, the level of dependency on the helper might be low or potentially facilitated.

Effects of the Black Queen trajectory: transformation of interactions and of the community

We propose that the BQH offers new perspectives as to how evolution can modulate community life. Notably: the emergence of a mutation within a population can (i) transform its interactions with other species and (ii) deepen community life and modify the global dynamics of the community.

The Black Queen as a way to elude competition

Niche partitioning (through environmental filtering or through species interactions sorting) and neutral processes (Hubbell, 2001) are classically acknowledged to drive community assemblies and explain diversity patterns. When competition occurs in spatially and temporally homogeneous environments, coexistence is mainly assumed to result from complementarity in resource use (Webb *et al*., 2002). In addition to these classical explanations of assembly rules we suggest that the transition from competition to dependency relationships may also be a driver of community structure.

In our simplified two-species model, the emergence of a helper-dependent mutant shifts competition toward coexistence. In this situation, helpers and beneficiaries will reach a state of equilibrium (Figure 2b) that bypasses exclusive competition.

The fact that long-term coexistence is reached through the evolution of dependency has already been demonstrated: de Mazancourt & Schwartz (2010) showed that resource trade enhances coexistence even if it decreases the abundance of one of the species (consistent with Figure 3b). In a similar way, Turcotte et al., (2012) showed in a context of waste-product exploitation that dependency increases the coexistence of species. Cross-feeding interactions

are also known to evolve in competitive environments (e.g. Friesen *et al*., 2004; Louca and Doebeli, 2015).

To put it simply, in a system where several species are in competition, if a beneficial dependency emerges between two species (through resource trade, waste-product or common good use), their coexistence will be optimized. Here, natural selection will favor the establishment of tighter relationships and confer an advantage to coexistence.

One change changes it all

In simulations where two species are able to sustain themselves irrespectively of the other's presence, an emerging loss-of-function mutant can invade the population and eventually replace the ancestral strain of that population(Figure 2b). We observe that the population density at steady state depends on life history traits such as the quantity of common good required(Figure 3a), lifespan(Figure 4a) and reproduction latency(Figure 4b). Increases and decreases in population sizes are attributed to a density dependence effect (Figures 3a, 4a and 4b) related to the parameters used in the model. The dynamics of the loss-of-function mutant and extinction of the original population will thus be dependent on such life history traits to reach a steady state.

Metabolic dependencies are potentially a major driver of species co-occurrence (Zelezniak *et al*., 2015). Until recently, the repercussions of such evolution at the community level were mostly overlooked, whereas it is now becoming evident that they are an integral part of community systems (Hairston *et al*., 2005; Johnson & Stinchombe 2007; Schoener, 2011) implying that evolutionary dynamics should systematically be taken into account when characterizing communities.

Dependence and consequences on the quantity of common goods produced

We propose that the quantity of common goods produced by helpers could impact the spatial dynamic of the entire community. In a scenario where the common good is abundant, the distribution of helpers and beneficiaries is expected to be homogeneous at steady state. Conversely in our simulation where the common good is produced at a limiting concentration, the dynamic invasion of space is uneven (MOVIE S1). The spatial distribution of beneficiaries, because of their dependency, is expected to adhere closely to the distribution of the helpers, inducing a local depletion of nutrients availability. Thus, the heterogeneous spatial 'aggregation' of (micro)organisms and resulting heterogeneity of nutrients availability will lead to temporal changes and patches displacement (MOVIE S1). Spatial and temporal heterogeneity may thus be a consequence of helper/beneficiary interaction.

Going further

The BQH, smoothing the way for long-lasting interactions?

Studies of co-evolution, taken in the broad sense where one organism evolves in relation to another, have tended to focus on the *acquisition* of traits and functions, with little attention given to the *loss* of functions in free-living organisms. Consequently, the mechanisms behind the emergence of a compensated loss of function remain unknown. It has been assumed that trait loss *[...] is only expected to evolve in long-term, stable co-evolutionary physiological relationships[...]* (Visser *et al*., 2010). However, we consider that the BQH offers a new approach: function loss, instead of resulting from long-lasting relationships, could actually be the cause of such long-lasting co-evolutionary relationships. Indeed, in the BQH, a 'passive' interaction (production and consumption of a common good) is at the basis of the emergence of tighter dependencies between two species.

We perceived that initially a mere coexistence of species within a community happens to be fortuitously beneficial for one of the species (via the redundancy in production of the common

good). Then if the beneficial conditions remain sufficiently stable over time, the (future) beneficiary species will tend to lose the compensated function. More than the trait loss itself (Ellers *et al*., 2012), we believe that the 'point of no return' in the shift from a facultative interaction to an obligatory dependency is the loss of gene(s) underlying the loss of function. In this case, genome reduction sets up the first steps for stable long-lasting metabolic interactions (Pande *et al*., 2013).

The loss of essential genes is widespread in free-living bacteria and most certainly at the root of inter-organisms networks (D'souza *et al*., 2014). In line with this, some vitamin exchanges are known to cement species connectedness within the community (Giovannoni, 2012) and it has also been inferred from the BQH model that cooperative interactions could rise "automatically" in this context (Sachs and Hollewell, 2014).

Corollary of the Black Queen Hypothesis

A species, by being dependent on another one, puts itself in a weakened position. If no helper species is around, the species cannot survive. What is more, such species can be considered as "accessory" for the community since they do not provide for the essential function. Admittedly, accessory species could be wiped out more easily than essential species since their loss will not have an immediate effect on the community. On the other hand, being able to handle essential functions, even if more costly, guarantees some degree of 'security'. First it allows for resistance against environmental changes or perturbation. Secondly, in a dependency context, helpers are indispensable, which will partly prevent them from being replaced by competing species (Nadell *et al.,* 2009). It is therefore of particular interest to keep, or even to acquire, the status of helper.

In their paper, Morris et al (2012) suggest that helpers are keystone species in the community. We propose a possible corollary of the BQH: species could benefit from ensuring

the status of helpers by having mutations that enhance the production of the common good, or more extremely, by acquiring genes producing the common good. The occurrence of mutation which enhances the common good production could also explain why some species turn into helpers while others become beneficiaries.

Horizontal gene transfer (HGT) is a common phenomenon in (micro)organisms (Mc Ginty *et al*., 2010) , often leading to the acquisition of functions and suggested to be an underlying mechanism of microbial cooperation (Smith, 2001). If the enhanced or acquired function leads to the development of a dependency interaction, then (micro)organisms possessing it may be better off, thanks to the key status acquired within the community. This substantiates the suggested BQH corollary, i.e., genome expansion for helpers who tend to embrace a generalist ecological status. Thus the BQH and its corollary invite a new interpretation of the networks of interactions and ecological status (i.e., generalist/specialist) of co-occurring organisms and their evolution.

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Supplementary information

Supplementary information is available at ISME J’s website

References

Cooper VS, Schneider D, Blot M, Lenski RE. (2001). Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of *Escherichia coli* B. *J Bacteriol* **183:**2834–2841.

De Mazancourt C, Schwartz MW. (2010). A resource ratio theory of cooperation. *Ecol. Let.* **13:**349–359.

Driscoll WW, Pepper JW, Pierson LS, Pierson EA. (2011). Spontaneous *gac* mutants of *Pseudomonas* biological control strains: Cheaters or mutualists? *Appl. Env. Microb.* **77:**7227–7235.

D'Souza G, Waschina S, Pande S, Bohl K, Kaleta C, Kost C. (2014). Less is more: Selective advantages can explain the prevalent loss of biosynthetic genes in bacteria. *Evolution* **68:**2559–2570.

Dufresne A, Garczarek L, Partensky F. (2005). Accelerated evolution associated with genome reduction in a free-living prokaryote. *Genome Biol.* **6:**R14.

Ellers J, Kiers TE, Currie CR, Mcdonald BR, Visser B. (2012). Ecological interactions drive evolutionary loss of traits. *Ecol. Let.* **15:**1071–1082.

Estes JA, Brashares JS, Power ME. (2013). Predicting and detecting reciprocity between Indirect Ecological Interactions and Evolution. *Am. Nat.* **181**:S76-S99.

Estrela S, Trisos CH, Brown SP. (2012). From metabolism to ecology: cross-feeding interactions shape the balance between polymicrobial conflict and mutualism. *Am. Nat.* **180:**566–576.

Estrela S, Morris JJ, Kerr B. (2015). Private benefits and metabolic conflicts shape the emergence of microbial interdependencies: The origins of microbial interdependencies. *Env. Microb.* **10**:1462-2920

Friesen ML, Saxer G, Travisano M, Doebeli M, Elena S. (2004). Experimental evidence for sympatric ecological diversification due to frequency-dependent competition in *Escherichia coli*. *Evolution* **58:**245–260.

Giovannoni SJ, Tripp HJ, Givan S, Podar M, Vergin KL, Baptista D, *et al.* (2005). Genome streamlining in a cosmopolitan oceanic bacterium. *Science* **309:**1242–1245.

Giovannoni SJ. (2012). Vitamins in the sea. *Proc. Natl. Acad. Sci.* **109:**13888–13889.

Giovannoni SJ, Thrash JC, Temperton B. (2014). Implications of streamlining theory for microbial ecology. *ISME J.* **8:**1–13.

Hairston NG, Ellner SP, Geber MA, Yoshida T, Fox JA. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Let.* **8:**1114–1127.

Hanson NW, Konwar KM, Hawley AK, Altman T, Karp PD, Hallam SJ. (2014). Metabolic pathways for the whole community. *BMC Genomics* **15:**619.

Hardin G. (1968). The Tragedy of the Commons. *Science* **162**:1243-1248 .

Hosoda K, Habuchi KM, Suzuki S, Miyazaki M, Takikawa G, Sakurai T, Kashiwagi A, *et al.* (2014). Adaptation of a cyanobacterium to a biochemically rich environment in experimental evolution as an initial step toward a chloroplast-like state. *PLoS ONE* **9:** e98337.

Hottes AK, Freddolino PL, Khare A, Donnell ZN1, Liu JC, Tavazoie S. (2013). Bacterial Adaptation through Loss of Function. *PLoS Genet.* **9:**e1003617.

Hubbell, S. P. (2001). The unified neutral theory of biodiversity and biogeography. *Princ. Univ. Press*. MPB-32**, 32**.

Hussa E, Goodrich-Blair H. (2013). It takes a village: ecological and fitness impacts of multipartite mutualism. *Annu. Rev. Microb.* **67:**161–178.

Johnson MT, Stinchcombe JR. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends Ecol. Evol.* **22:**250–257.

Kreft JU, Bonhoeffer S. (2005). The evolution of groups of cooperating bacteria and the growth rate versus yield trade-off. *Microbiology* **151:**637–641.

Lahti DC, Johnson NA, Ajie BC, Otto SP, Hendry AP, Blumstein DT, *et al.* (2009). Relaxed selection in the wild. *Trends Ecol. Evol.* **24:**487–496.

Lee MC, Marx CJ. (2012). Repeated, selection-driven genome reduction of accessory genes in experimental populations. *PLoS Genet.* **8:**2–9.

Louca S, Doebeli M. (2015). Calibration and analysis of genome-based models for microbial ecology. *eLife* **4:**e08208.

Luo H, Swan BK, Stepanauskas R, Hughes AL, Moran MA. (2014). Evolutionary analysis of a streamlined lineage of surface ocean Roseobacters. *ISME J.* **8:**1428–1439.

McCutcheon JP, Moran NA. (2011). Extreme genome reduction in symbiotic bacteria. *Nat. Rev. Microb.* **10:**13–26.

McGinty SE, Rankin DJ, Brown SP. (2011). Horizontal gene transfer and the evolution of bacterial cooperation. *Evolution* **65:**21–32.

Morris JJ, Lenski RE, Zinser ER. (2012). The black queen hypothesis: Evolution of dependencies through adaptive gene loss. *MBio* **3:**e00036-12.

Morris JJ, Papoulis SE, Lenski RE. (2014). Coexistence of evolving bacteria stabilized by a shared black queen function: experimental evolution of a black queen community. *Evolution* **68**:2960-2971.

Nadell CD, Xavier JB, Foster KR. (2009). The sociobiology of biofilms. *FEMS Microb. Rev.* **33:**206–224.

Ochman H, Moran N. (2001). Genes lost and genes found: evolution of bacterial pathogenesis and symbiosis. *Science* **292:**1096–1099.

Pande S, Merker H, Bohl K, Reichelt M, Schuster S, de Figueiredo L, *et al.* (2014). Fitness and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria. *ISME J.* **8:**953–962.

Partensky F, Hess WR, Vaulot D. (1999). Prochlorococcus, a marine photosynthetic prokaryote of global significance. *Microb. Mol. Biol. Rev.* **63:**106–127.

Porter ML, Crandall KA. (2003). Lost along the way: The significance of evolution in reverse. *Trends Ecol. Evol.* **18:**541–547.

Richards TA, Talbot NJ. (2013). Horizontal gene transfer in osmotrophs: playing with public goods. *Nat. Rev. Microb.* **11:**720–727.

Sachs JL, Hollowell C. (2012). The origins of cooperative bacterial communities. *MBio* **3**.

Schoener TW. (2011). The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* **331:**426–429.

Smith J. (2001). The social evolution of bacterial pathogenesis. *Proc. R. Soc. Lond. B* **268:**61–69.

Turcotte MM, Corrin MSC, Johnson MTJ. (2012). Adaptive Evolution in Ecological Communities. *PLoS Biol.* **10:**e1001332.

Van Valen L. (1973). A new evolutionary law. *Evol. Theory* **1:**1–30.

Visser B, Le Lann C, den Blanken FJ, Harvey JF, van Alphen JJM, Ellers J. (2010). Loss of lipid synthesis as an evolutionary consequence of a parasitic lifestyle. *Proc. Natl. Acad. Sci. USA* **107:**8677–8682.

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ. (2002). Phylogenies and community ecology. *Annu. Rev. Ecol. Syst.* **33:**475–505.

Wolf YI, Koonin EV. (2013). Genome reduction as the dominant mode of evolution: Prospects & Overviews. *BioEssays* **35:**829–837

Zelezniak A, Andrejev S, Ponomarova O, Mende DR, Bork P, Patil KR. (2015). Metabolic dependencies drive species co-occurrence in diverse microbial communities. *Proc. Natl. Acad. Sci. USA* **112:**6449–6454.

FIGURES LEGENDS:

Figure 1| Overview of the Black Queen Hypothesis.

The yellow and green spots represent 2 types of microorganisms, namely A and B1 in the next figures, both producing a common good. A mutant which has lost the capacity to produce the common good is shown in blue (B2 in the next figures). The shade of the background (dark grey) expresses the concentration of the common good in the environment. **(a)** Initial state: A and B are present **(b)** as the common good is extensively produced in the environment, a mutant strain (B2, blue) no longer able to produce the common good emerges, it is dependent (i.e., beneficiary) on other microorganisms producing it (i.e., helpers) **(c)** as the beneficiary's fitness is improved (no energy invested in the production), it invades the population **(d)** and a new equilibrium is reached in the community between the helpers (yellow) and beneficiaries (blue) which supplanted their ancestors.

Figure inspired from Bjørn Østman, 2012, http://pleiotropy.fieldofscience.com/2012/05/black-queen-hypothesis.html.

Figure 2| Trajectories for two species populations (A and B1) when a loss of function mutant (B2) arises in one population.

The trajectories shown represent a single simulation that measures the population of each species over time as defined by the species' rules given in the Supplementary Information. **(a)** As the fitter mutants B2 (blue) emerge within their original population B1 (green), they will increase to the detriment of this original population. However, because the mutants still depend on the common good produced by the helpers (B1s), they can only spread if there are enough B1s present to produce the common good, leading to an equilibrium state of helper and mutant populations. **(b)** When the fitter mutants arise from their original population B1, if another species (A, in yellow) also produces the common good, then the B2 population will not be exclusively dependent on the B1s, and (B2s) will entirely supplant the B1s if the B1s provide enough of the common good to sustain the B2s.

Figure 3| Steady state populations of the original population B1 (helper) and of the fitter mutant B2 (beneficiary) with changing parameter values of density and of quantity of common good required.

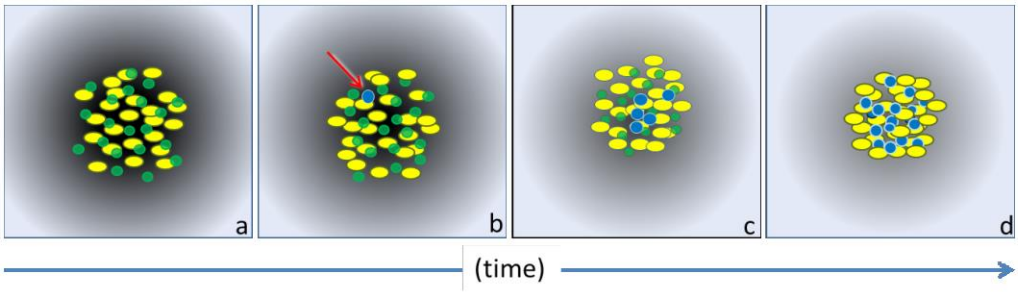
The population of B1s in green (the original population which became a helper population) and B2s in blue (the beneficial mutants) is shown after they have reached equilibrium. Fifty simulation replicates were performed for each modality and data were collected after 500 time-steps (see supplementary information). The default values for the fixed parameters were in **(a)** and **(b)** reproduction latency=3, lifespan=3, in (**a**) only, minimum of common good required=5 and in **(b)** only density=3. Both (**a**) the density value (in arbitrary units, corresponding to the upper limit of individuals living in a small radius) and (**b**) the quantity of required common good by beneficiaries (arbitrary units) are altered to see how the equilibrium changes. Whiskers show confidence intervals (α=0.5) of the means.

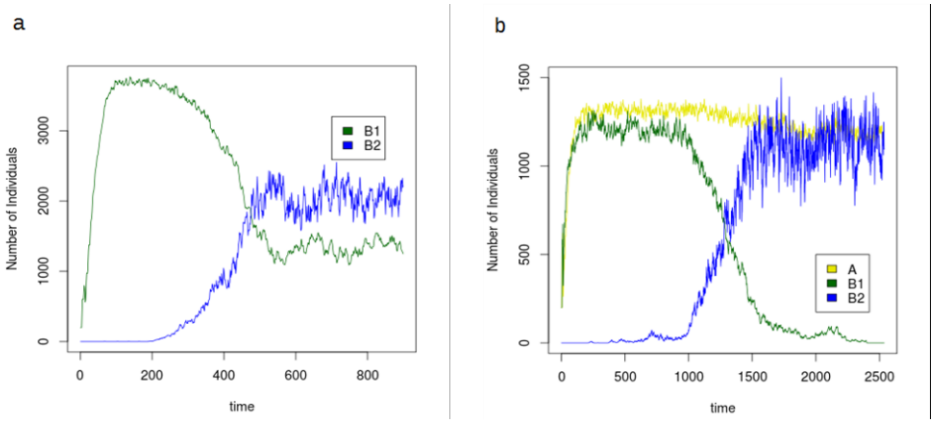
(a) The density is indirectly representative of the nutrient resource available: if more resource is available, more organisms can live together on a given surface unit. Because the beneficiaries B2 are fitter, they will supplant the B1s when more nutrients are available (Kruskal-Wallis test, $P<2.10^{-16}$) while B1 density hardly changes (Kruskal-Wallis test, $P= 0.0191$). However, because the B2s are dependent on the common good, they can only spread if there is enough common good produced, thus the helpers' population (B1) cannot be excluded **(b)** When the minimum quantity of common good required by beneficiaries B2s is higher (i.e., the mutants have a greater need in common good) the B2s will be more dependent on the helpers B1. As the need in common good for the B2s increases, their population density at steady state is lowered (Kruskal-Wallis test, $P<2.10^{-16}$) and conversely for the B1 (Kruskal-Wallis test, $P<2.10^{-16}$).

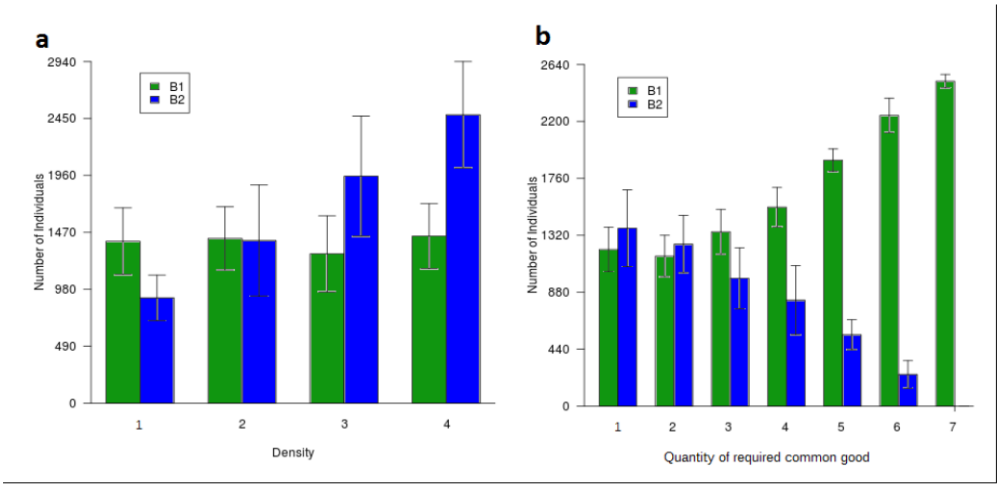
Figure 4| Steady state populations of the original population B1(helper) and of the fitter mutant B2 (beneficiary) with changing values of lifespan and reproduction latency parameters, a and b respectively.

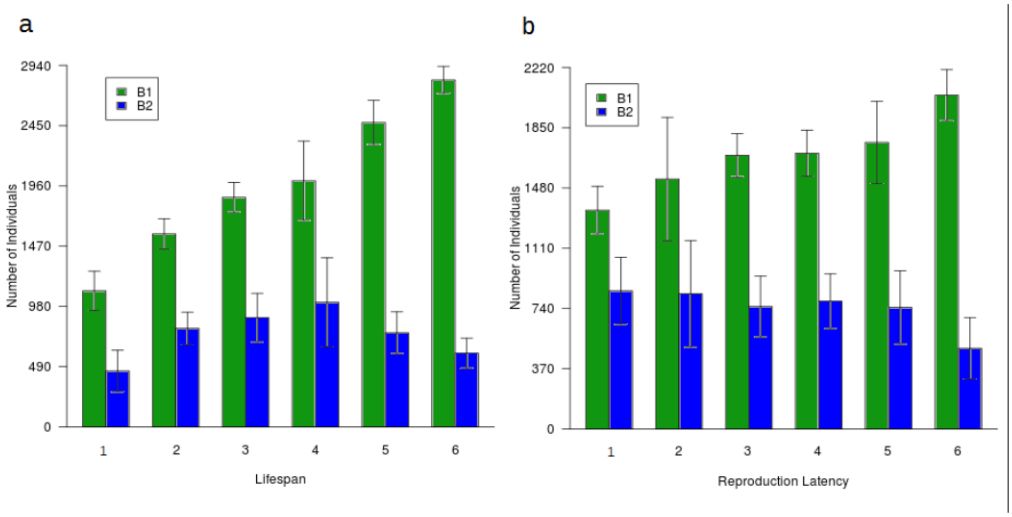
The population of helpers (B1s) and beneficiaries (B2s) is shown after they have reached equilibrium. The lifespan (corresponding to the time available for reproduction, in arbitrary units) and the reproduction latency (the time before individuals can reproduce, in arbitrary units) were altered to see how the equilibrium changed. The population(s?) of helpers (B1) and beneficiaries (B2) were sampled at 50 different time-points after introducing the mutant (B2) and when an equilibrium state (500 time-steps) was reached for each set of parameters. Each bar shows the mean of the 50 time-points and the whiskers represent the 95% confidence interval. The default values of the fixed parameters were: lifespan=3, reproduction latency=3, density=3, the minimum quantity of common good required by beneficiaries=5. **(a)** With increasing lifespan (Kruskal-Wallis test, $P<2.10^{-16}$) a B1 individual is guaranteed to have enough common good in the final time-steps of its life. A B2 individual, however, needs to coexist with a B1 on the same patch for there to be enough common good. That is, the chances of a beneficiary individual reproducing decrease because of the

greater dependency on helpers. **(b)** Similar reasoning as for lifespan holds for the reproduction latency.


a
b
c
d
(time)


a
Number of Individuals
0
1000
2000
3000
0
200
400
600
800
time
B1
B2
b
Number of Individuals
0
500
1000
1500
0
500
1000
1500
2000
2500
time
A
B1
B2


a
B1
B2
Number of Individuals
2940
2450
1960
1470
980
490
0
1
2
3
4
Density
b
B1
B2
Number of Individuals
2640
2200
1760
1320
880
440
0
1
2
3
4
5
6
7
Quantity of required common good


a
b
B1
B2
Number of Individuals
2940
2450
1960
1470
980
490
0
2220
1850
1480
1110
740
370
1
2
3
4
5
6
Lifespan
Reproduction Latency